

What is claimed is:

- 5 1. A method for immunizing an individual to prevent
disease caused by a gram-negative bacterial pathogen,
the method comprising vaccinating the individual with a
prophylactically effective amount of a vaccine
formulation comprising an active ingredient selected
10 from the group consisting of an *htrB* mutant of said
gram-negative bacterial pathogen, endotoxin isolated
from the *htrB* mutant of said gram-negative bacterial
pathogen, endotoxin isolated from the *htrB* mutant of
said gram-negative bacterial pathogen said endotoxin
15 conjugated to a carrier protein, and an *htrB* mutant of
said gram-negative bacterial pathogen which has been
genetically engineered to express at least one
heterologous vaccine antigen; wherein said *htrB* mutant
lacks one or more secondary acyl chains of lipid A
20 contained in the gram-negative bacterial pathogen
resulting in substantially reduced toxicity when
compared to lipid A of the gram-negative bacterial
pathogen.
- 25 2. The method of claim 1, wherein the individual is a
human, and the vaccine formulation is introduced by a
route of administration selected from the group
consisting of intradermal, intramuscular,
intraperitoneal, intravenous, subcutaneous, ocular,
30 intranasal, and oral administration.
3. The method of claim 2, wherein the vaccine
formulation comprises an active ingredient consisting
essentially of an *htrB* mutant of said gram-negative
35 bacterial pathogen.

4. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen.
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5. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.
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6. The method of claim 2, wherein the vaccine formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a microbial pathogen.
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7. The method of claim 2, wherein the vaccine formulation further comprises a physiological carrier and an adjuvant.
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8. The method of claim 1, wherein the individual is an animal, and the vaccine formulation is introduced by a route of administration selected from the group consisting of intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration.
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9. The method of claim 8, wherein the vaccine formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen.
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10. The method of claim 8, wherein the vaccine formulation comprises an active ingredient consisting
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essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen.

11. The method of claim 8, wherein the vaccine
5 formulation comprises an active ingredient consisting essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.
12. The method of claim 8, wherein the vaccine
10 formulation comprises an active ingredient consisting essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a
15 microbial pathogen.
13. The method according to claim 8, wherein the *htrB* mutant of said gram-negative bacterial pathogen is administered orally as an additive to animal feed.
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14. The method according to claim 12, wherein the *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous antigen from a microbial pathogen is
25 administered orally as an additive to animal feed.
15. The method of claim 8, wherein the vaccine formulation further comprises a physiological carrier and an adjuvant.
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16. A vaccine formulation comprising an active ingredient selected from the group consisting of an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative
35 bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen said

endotoxin conjugated to a carrier protein, and an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at least one heterologous vaccine antigen; wherein said *htrB* mutant
5 lacks one or more secondary acyl chains of lipid A contained in the gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the gram-negative bacterial pathogen.

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17. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of an *htrB* mutant of said gram-negative bacterial pathogen.

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18. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen. B

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19. The vaccine formulation of claim 16, wherein the active ingredient consists essentially of endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, wherein the isolated endotoxin is conjugated to a carrier protein.

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20. The vaccine formulation according to claim 16, wherein the active ingredient consists essentially of an *htrB* mutant of said gram-negative bacterial pathogen which has been genetically engineered to express at
30 least one heterologous antigen from a microbial pathogen.

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21. The vaccine formulation according to claim 16, further comprising a physiological carrier and an adjuvant.

Sub 7
22. A method of making in a gram-negative bacterial pathogen a mutant endotoxin of substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, the method comprising
5 mutating an *htrB* gene within the gram-negative bacterial pathogen, wherein said mutation causes a phenotype of a resultant *htrB* mutant characterized by a mutant endotoxin lacking one or more secondary acyl chains of
10 lipid A contained in the wild type gram-negative bacterial pathogen.

23. A mutant endotoxin of substantially reduced toxicity, made according to the method of claim 22, wherein the mutant endotoxin having substantially
15 reduced toxicity was purified from the *htrB* mutant by a process selected from the group consisting of a phenol/water extraction, and a protease digestion; and wherein the purified mutant endotoxin having
20 substantially reduced toxicity is used to generate endotoxin-specific antibodies.

24. The mutant endotoxin according to claim 23, further comprising conjugation to a carrier protein.

25. A mutant endotoxin of substantially reduced toxicity, made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, further comprising conjugation to a carrier protein.

27. A method of making an *htrB* mutant of a wild type gram-negative bacterial pathogen wherein the *htrB* mutant has substantially reduced toxicity when compared to the wild type gram-negative bacterial pathogen, the method
35 comprising mutating an *htrB* gene within the gram-negative bacterial pathogen, wherein said mutation

causes a phenotype of a resultant *htrB* mutant characterized by endotoxin lacking at least one secondary acyl chain on lipid A contained in the wild type gram-negative bacterial pathogen.

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28. A mutant gram-negative bacterial pathogen of substantially reduced toxicity, made according to the method of claim 27, wherein the gram-negative bacterial pathogen having substantially reduced toxicity is used
10 to generate endotoxin-specific antibodies.

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29. A method for producing endotoxin-specific antisera for a use selected from the group consisting of in diagnostic assays, and for passive immunization, the method comprises immunizing an individual with a vaccine formulation comprising an active ingredient selected from the group consisting of an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen, and endotoxin isolated from the *htrB* mutant of said gram-negative bacterial pathogen said endotoxin conjugated to a carrier protein; and collecting antibody produced from said immunized individual; wherein said *htrB* mutant lacks one or more secondary acyl chains of
20 lipid A contained in the wild type gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.
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Add C47

Add D3

add H4